REPORT DOCUMENTATION PAGE

AFRL-SR-AR-TR-03-

0105

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1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
		01 Jul 02 - 31 Dec 02 FINAL

4. TITLE AND SUBTITLE
Equipment for high frequency measurements for gene expression studies

5. FUNDING NUMBERS
61102F
2301/ZE

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NE
4015 WILSON BLVD
SUITE 713
ARLINGTON VA 22203

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
F49620-02-1-0327

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
APPROVED FOR PUBLIC RELEASE, DISTRIBUTION UNLIMITED

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*
This award has enabled us to obtain necessary equipment for our research supported by the MURI 02 grant on the RF bio-effects for homeland defense.

20030508 117

14. SUBJECT TERMS			15. NUMBER OF PAGES
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

Final Report of the Air Force Equipment Grant

Equipment for High Frequency Measurements for Gene Expression Studies

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March 26, 2003

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Introduction

The equipment grant, "Equipment for high frequency measurements for gene expression studies," awarded by the Air Force Office of Scientific Research was used to purchase the equipment needed for research supported by the MURI 02 grant on the RF Bio-Effects for Homeland Defense. The equipment purchased was mainly for 1) the construction of RF applicators and a network analyzer for instrumentation, 2) gene expression analysis using the microarray technology, and 3) upgrading a DNA sequencer for gene expression analysis using the SAGE-GLGI technology.

Equipment Purchased

I. For RF applicators and network analyzer for instrumentation

Item	cost
Microwave network analyzer and accessories (Agilent)	73,516.16
Fluoropotic probe sensor and accessories (Luxtron)	16,082.30
Henry 2KD Classic, Predriver, and accessories (Henry Radio)	3,905.00
Isolator and accessories (Cober-Muegge)	3,851.46
Modulation power meter with sensors (Nolateck)	3,030.00
Circulating water Bath (Fisher)	2,533.33
Pulse generator (Quantium Composers)	1,036.00
Thermocouple and temperature control (Omega)	511.70
BTX Safety Stand and accessories (Fisher)	576.29
Air regulator and specronic UV (Fisher)	423.54
Flowmeter (Fisher)	170.00
Subtotal	**105,635.78**

II. For microarray analysis

Item	cost
Storm phosphoimager and workstation (Amersham)	38,228.00
Altasimage and accessories (Clontech)	4,740.21
Hybridization incubator (Fisher)	2,828.05
Screen and cassette (Amersham)	863.80
Subtotal	**46,660.06**

III. For SAGE-GLGI analysis

Item	cost
MegaBace upgrade	50,000.00
LPA tubes (Amersham)	1,184.10
Window 2000 (Follet)	319.00
Subtotal	**51,503.10**

Total: $203798.94 – $4000* = $199,798.94 (Net Expenses)

*Equipment charges transferred to MURI 02 grant.

Work Accomplished (In Conjunction with MURI 02 Grant)

I. Non-thermal 2.45 GHz system (completed)

- The cell culture is housed in a 25 ml flask having its bottom surface directly in contact with the bottom surface of the waveguide.
- The bottom surface temperature is maintained at 37± 0.1 °C using a water channel fastened on the outside surface of the waveguide. The waveguide is terminated with a matched load to minimize standing waves.
- Pulse width and off-time are independently controlled.
- Three samplers and three power meters are used to measure the power absorbed.
- The magnitude of the electric field at the cell monolayer layer can be determined using an electromagnetic finite element software package, and the value of incident power.
- One type of output from the software is a planar distribution of electric field magnitude that is in the form of a contour plot. The contour areas correspond with a range of electric field magnitude that can be readily exported into MATLAB® and analyzed.

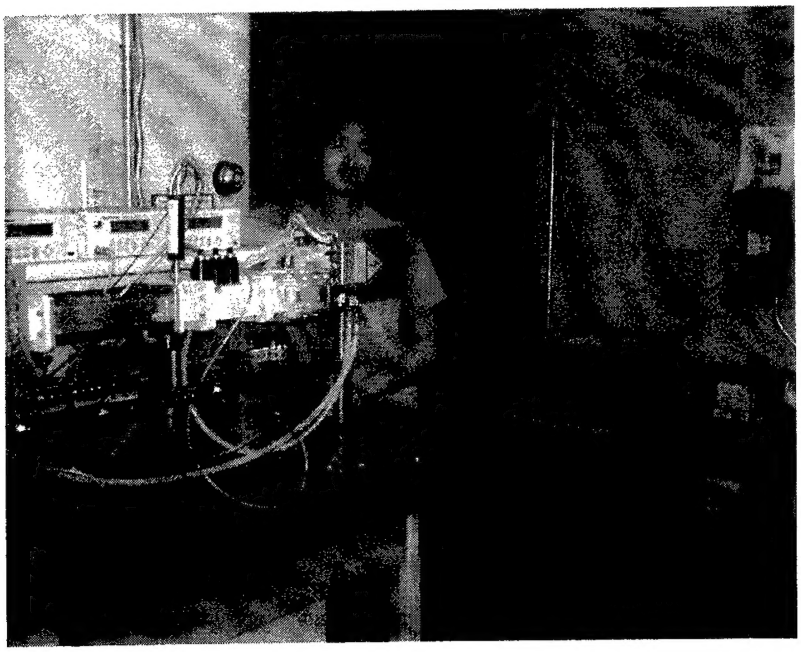


Fig.1. Overall view of the 2.45 GHz microwave system

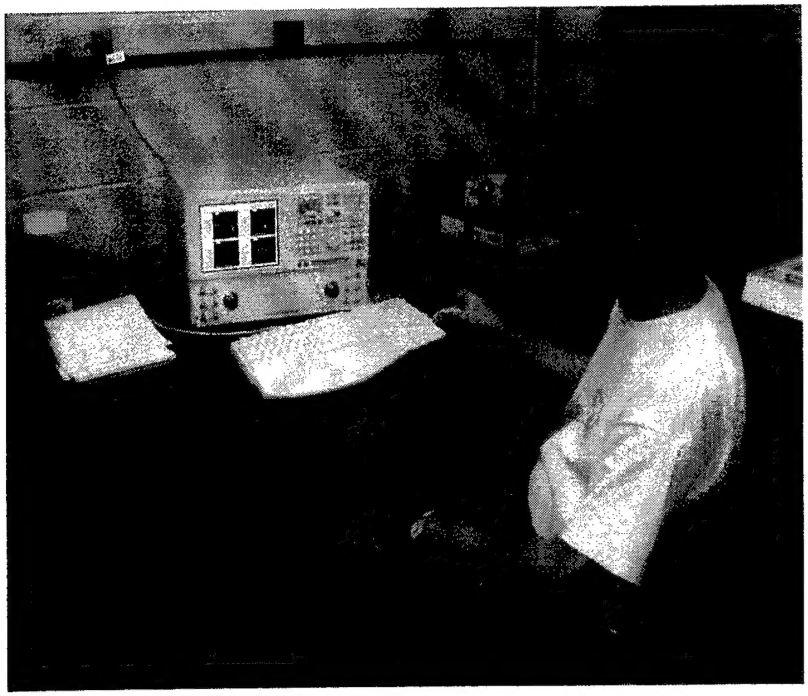


Fig. 2. Network analyzer for Instrumentation

II. Non-thermal 3.5-30 MHz system (in progress)

- Matched network to cuvette
- Maximum electric field in culture: 500 V/cm
- Pulsed operation

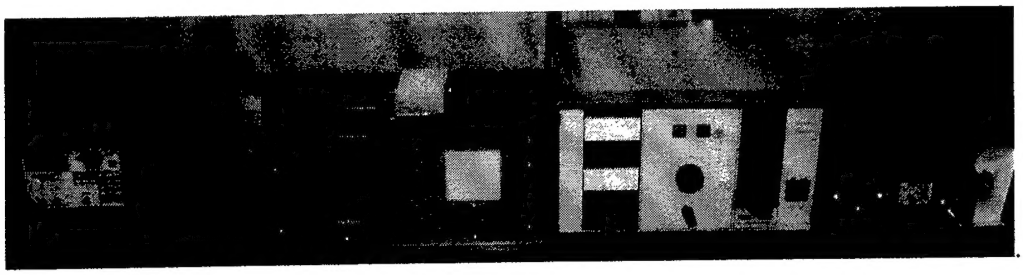


Fig. 3. General view of low frequency amplifier system

III. Analysis of gene expression (in progress)

A. RF exposure experiments (conducted at Washington University in collaboration with Joseph Roti Roti's group)

Human HL60:	2 hours:	837 MHz (cw)
		2.45 GHz (cw)
	21 hours:	837 MHz (cw)
		2.45 GHz ((cw)
Escherichia. coli:	21 hours	2.45 GHz (cw)
Bacillus subtilis	2 hours8	37 GHz (cw)
	21 hours	2.45 GHz (cw)

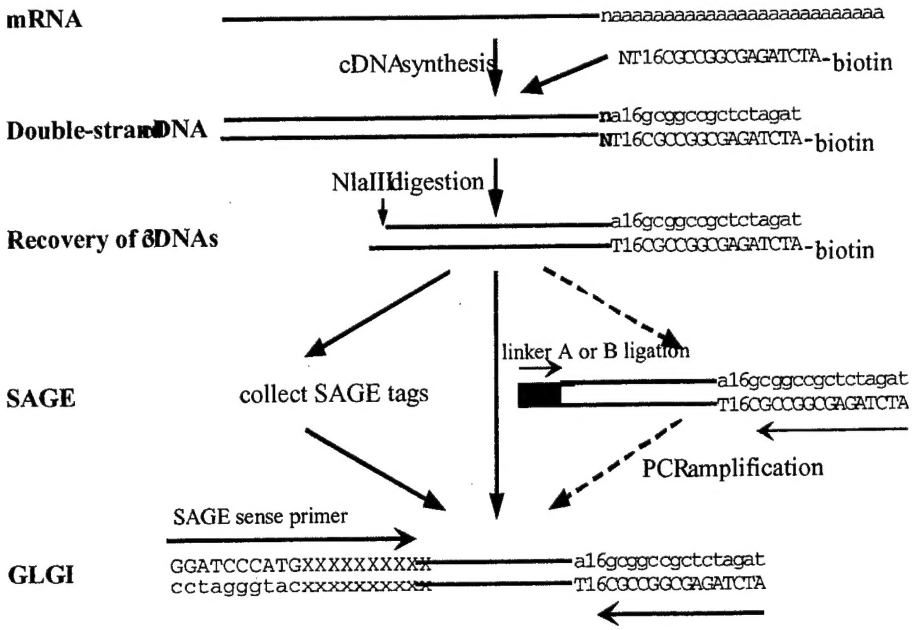
B. Gene expression analysis using microarray technology (in progress)

- Microarrays used for human genomic analysis: The12K plastic microarray contains 12,000 long oligos of known human genes, including house keeping genes, proto-oncogenes, tumor suppressor genes, transcription factor genes, stressed genes, etc.
- Microarrays used for bacteria: *Escherichia. coli* gene array (4000 genes)
  Bacillus subtilis gene array (4000 genes)
- Isolation of total RNA from controls and RF exposed cells: HL60, *E. coli*, and *B. subtilis*
- Probe preparation
- cDNA synthesis
- Hybridization
- Array analysis

Fig.4. Storm 840: Phosphoimager for microarray scanning

C. Gene expression analysis using SAGE-GLGI technology (in progress)

The following diagram shows the basic steps of SAGE followed by GLGI (Generation of longer 3' ESTs from SAGE tags for gene identification).



- The HL60 human promyelocytic cell line was exposed to 837 MHz (10w/kg) for 2 hours using an RTC system.
- The cell cultures were gassed with 5% CO_2 and grew in the flasks. The cell density was 10^6 cells/ml in a total volume of 40 ml. During the exposure period, the temperature was monitored and maintained at 37°C (± ≤0.5°C).
- Immediately after exposure, the cell pellet was treated with a lysis buffer and total RNA was isolated using the guanidine thiocyanate/acid phenol:chloroform extraction method. About 50 µg of the total RNA were used for microarray analysis and 5 µg of the total RNA were used for SAGE analysis.
- SAGE libraries are now being generated, and the SAGE tags that show difference in expression between the control and the exposed cells will be further characterized with GLGI.

Fig. 5. MegaBace DNA Sequencer